SPGR Sub-Project Completion Report (PCR)

Somatic cell count and bacterial characterisation as tools for diagnosis and identification of risk factors of bovine mastitis

Duration: August 2012 to April 2014

Executing Organization

Department of Medicine
Faculty of Veterinary Science
Bangladesh Agricultural University
Mymensingh-2202

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List of Abbreviations

BARC	:	Bangladesh Agricultural Research Council
BAU	:	Bangladesh Agricultural University
BAURES	:	Bangladesh Agricultural University Research System
BCC	:	Bacterial Colony Count
BCS	:	Body Condition Score
CDVF	:	Community-based Dairy Veterinary Foundation
CI	:	Confidence Interval
CMT	:	California Mastitis Test
CNS	:	Coagulase Negative *Staphylococcus aureus*
FAPAD	:	Foreign Aided Project Audit Directorate
LoA	:	Letter of Agreement
OR	:	Odds Ratio
SCC	:	Somatic Cell Count
SCM	:	Subclinical Mastitis
SoE	:	Statement of Expenditures
UGC	:	University Grants Commission of Bangladesh

Executive Summary

This report summarizes the technical as well as financial aspects of the project no. 379, entitled "Somatic cell count and bacterial characterisation as tools for diagnosis and identification of risk factors of bovine mastitis" for the entire project period from August 2012 to April 2014. The major activities successfully completed were a) adoption and standardization of somatic cell count in milk samples, b) farm investigation and data collection (in cross-sectional study 399 dairy farms comprising 931 cows, and in a case-control study 117 case and 117 control farms were investigated), c) milk samples collection and bacteriological analysis, d) training of veterinarians of the project areas on Dairy Cattle Health and Production Management, and e) workshop on prevention and control of mastitis in dairy cows with the participation of learned teaching staffs, veterinarians, PhD and MS Fellows, project implementing personnel and BAURES authority. The farm-level and cow-level prevalence of subclinical mastitis (SCM) was 29.3% and 16.5%, respectively. Analysis of risk factors revealed 11 variables as potential independent farm-level risk factors for SCM.). These were absence of and insufficient sunlight, floor component (soil), dirty floor condition, no use of antiseptic after floor cleaning, infrequent floor cleaning (once or twice daily), different milking man, cows not milked sequentially, history of clinical mastitis during last year, cows not grazed during day, wipes udder and teat before milking and age of shed above 5 years, which had the strongest association with SCM with high statistical significance ($p \leq 0.05$). As cow-level risk factors, five independent risk factors for SCM were identified. These were breed, parity, cylindrical type of teat, pendulous type of udder and history of previous clinical mastitis which had the strongest association with SCM with high statistical significance ($p<0.05$). The major achievements are i) establishment of early diagnostic facilities of SCM in lactating dairy cows by automatic somatic cell counter, ii) major and minor pathogens causing SCM are identified and correlated with somatic cell count, iii) prevalence and risk factors of SCM in lactating dairy cows are known, iv) skills on dairy cattle health and production management of the veterinarians of project areas developed, v) mastitis control guidelines (booklet and leaflet) are formulated and distributed to the smallholder dairy farmers in the project areas.

1. **Sub-Project title:** Somatic cell count and bacterial characterisation as tools for diagnosis and identification of risk factors of bovine mastitis

2. **Principal Investigator:** Dr. Md. Taohidul Islam

3. **Full address with phone, cell and e-mail:**

Associate Professor
Department of Medicine
Bangladesh Agricultural University, Mymensingh
Phone- 01912-910338
Email- taohid.bau@gmail.com

4. **Duration of the sub-project:** 01 August 2012 to 30 April 2014

5. **Date of approval** (by the Executive Council/signing of LoA): 01 August 2012

6. Total approved Budget (Taka): 46,96,640.00
Total fund released (Tk): 46,96,640.00
Total fund Spent (Tk): 46,96,640.00
Unspent/balance fund (Tk.): 0.00
Reason for the balance: N/A

7. **Justification of undertaking the sub-project:**

Mastitis is the most common and costly production disease in dairying worldwide (Seegers et al., 2003; Halasa et al., 2007). It causes losses due to reduction of milk yield and cost of therapy and other inputs mobilised to control mastitis. The actual losses from mastitis in Bangladesh are not known yet. Worldwide, published estimates of the economic losses of clinical mastitis range from €61 to €97 per cow on a farm; however, large differences between farms do exist. For instances, in the Netherlands, losses due to clinical and subclinical mastitis varied between €17 and €198 per cow per year (Hogeveen et al., 2011). Mastitis deteriorate milk quality, as it decreases nutrient contents, alters flavour and increases microbial loads in milk and encourage drug residues from antibiotic therapy. Research data on mastitis in Bangladesh are scanty. Such data are essential needs to formulate a national mastitis control programme. Chowdhury (2011) performed a cross sectional study in dry and wet season in different areas of Bangladesh to identify the prevalence and risk factors of mastitis, and the seasonal distribution and microbial pattern of mastitis. In studies mentioned above, the California Mastitis Test (CMT) was used to identify mastitis cows; however, CMT is an indirect method and it gives only first hand information about the mastitis. On the other hand, Somatic Cell Count (SCC) is more direct and specific and it gives a clear reflection on the status of udder health of dairy animals. By using research data, countries with developed dairy industries have set the SCC threshold of 200,000 cells/mL for normal milk. However, cow breed and management practices in those countries are different. In Bangladesh, the dairy animals are indigenous and crossbred cows and buffaloes and the SCC in their milk is not known, which is likely to differ. Therefore, it is necessary to determine the SCC in milk of both normal and mastitis cows and buffaloes in Bangladesh.

In context to the expanding dairy industry in Bangladesh, it is crucial to develop a quick mastitis diagnosis scheme and control programme. Mastitis control is very important not only to reduce its incidence in dairy animals but also to reduce public health hazards. The latter is caused by consumption of infected milk and milk contaminated by drug residues that comes from the treatment of clinical mastitis. Somatic cell count in individual milk and bulk tank milk is the quick and established method to identify subclinical and clinical mastitis and bacterial colony count and colony type determination is the procedure for bacterial characterization. Somatic cell count together with bacterial colony count (BCC) in normal and mastitis milk with regards to data on risk factors of mastitis will help us to develop a more effective guideline for a nationwide mastitis control programme. Further, such information will be used for educating veterinary students and for dissemination among veterinarians and extension workers.

8. Sub-Project Objectives:

a. Adoption and standardisation of techniques for somatic cell count (SCC)
b. Isolation and identification of bacterial species and colony count and relating that with SCC
c. Identification of risk factors for mastitis
d. Formulation of a mastitis control strategy by relating data on SCC and bacterial colony count (BCC) with identified risk factors

9. Methodology followed in conducting research/investigation

9.1 Study areas and period

The study is being carried out in Mymensingh (Trishal), Sirajgonj (Ullapara), Satkhira (Sadar, Debhata, Ashashuni), and Chittagong (Potiya) districts. The smallholder dairy farmers in these areas are being provided with subsidized productivity veterinary services by the Community-based Dairy Veterinary Foundation (CDVF). The project utilises CDVF facilities for its implementation. Though the LoA of the project signed in August 2012, the research activities at the field and laboratory started from October 2012 and completed in November 2013.

9.2 Study population, design and sample size

A cross sectional study was carried out in randomly selected 399 dairy farms (931 lactating cows) from a list of 2800 farms in the aforesaid areas to have the baseline prevalence data as well as a comprehensive list of case and control cows/farms. A cow was considered as a case if one of the four quarters was positive for subclinical mastitis (SCM). And a farm was considered as a case farm when at least one cow suffered from SCM. On the other hand, a farm was considered as control farm where not a single cow affected with SCM. Based on the findings of cross-sectional study, 117 farms were case farms and 154 cows were case cows. Matching the location of farms, 117 control farms and 308 control cows were randomly selected from a list of 282 control farms and 777 control cows, respectively (Table 1).

Table 1: Sample size for two types of study at four study areas

District	Cross-sectional study		Case-control study			
			Case/control farms (1:1 ratio)		Case/control cows (1:2 ratio)	
	No. of farms	No. of cows	Case farms	Control farms	Case cows	Control cows
Chittagong	88	380	34	34	58	116
Mymensingh	11	28	5	5	5	10
Satkhira	248	450	73	73	86	172
Sirajgonj	52	73	5	5	5	10
Total	399	931	117	117	154	308

9.3 Inclusion and exclusion criteria

A farm having at least one lactating cow was considered for inclusion in the study. All the lactating cows present in each farm at the day of farm visit with no visible abnormality of the milk and udder were included in the study. Cows in the first 14 days of lactation were excluded from this study. Cows experienced with clinical mastitis during study lactation were excluded from investigation.

9.4 Farm data collection

A pretested questionnaire (Annex I) was administered to collect information about each farm's location, housing and farm management in general, feeding, milking procedures, milk production, medication, specific cow data, etc. A farm was surveyed once in a point in time. Two trained MS veterinary students and two field assistants were directly involved in the collection of data (Fig. 1-2).

Fig. 1-2. Collection of data from smallholder dairy farmers.

9.5 Adoption and standardisation of somatic cell count in cows' milk

Individual milk samples from 50 cows were collected. Serial dilution of the milk samples was made and somatic cell in each sample and different dilutions were counted by NucleoCounter SCC-100 somatic cell counter (Fig. 3a-b) as well as by direct microscopic examinations and data on SCC from NucleoCounter were matched and verified for standardisation.

Fig. 3a-b. NucleoCounter SCC-100 Somatic Cell Counter.

9.6 Collection of milk samples for somatic cell count

All quarter milk samples (n=3724) from 931 cows were collected conveniently either before or after the collection of data after wiping the teat by cotton soaked with 70 % alcohol (ethanol). First two milk streams were discarded, and then 10 ml of milk was taken from each quarter into labelled sterilized test tubes with rubber cap and subjected to somatic cell counting by automatic machine (NucleoCounter SCC-100, Chemometec, Denmark) (Fig. 4a-b).

Fig. 4a. Preparation of milk samples for somatic cell counting by automatic machine.

Fig. 4b. Prepared milk samples are placed into the automatic somatic cell counter.

9.7 Diagnosis of SCM

Diagnosis of SCM was based on the somatic cell count (SCC) and findings of examination of cow's udder and milk. A count of somatic cell >200,000/ml of milk was considered positive for subclinical mastitis with no visible abnormalities in cows' udder and milk.

9.8 Bacteriological analysis

A total of 100 milk samples (50 from case and 50 from control cows) were collected and transported on ice to the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh for bacteriological studies. Bacteria from the milk samples were isolated and identified according to diagnostic schemes published by the National Mastitis Council (Hogan et al. 1999) in the *Laboratory and Field Handbook on Bovine Mastitis*. Briefly, samples were cultured in enriched media at 37^0C for overnight after processing. Decimal dilutions were made in physiological saline and plated on to agar plates. The plates were incubated at 37^0 C for overnight. After incubation and examination of colony characteristics, a portion of individual colonies of different types were streaked on appropriate selective media (EMB, SS, Brilliant Green agar etc) to get the bacteria in pure culture. Individual pure culture was frozen at -80^0C in tryptone soya broth with 20% glycerol, if not processed immediately. All isolates were cultured in selective media, stained by Gram's staining and tested for biochemical activities by available standard methods for confirmatory identification of bacteria. Bacteriological data were recorded by using Microsoft Excel Work Books. Important bacterial isolates were stored at -86^0C for future use and referencing.

9.9 Data analysis

Descriptive statistics as well as basic univariate data analyses to check for the association between factors of interest and the outcome status (subclinical mastitis positive) were performed according to Hosmer and Lemeshow (2000) and Dohoo et al. (2003) by using MS Excel and SPSS 17.0. For the identification of risk factors for mastitis, uni- and multivariate logistic regression models were constructed and run within the stepwise (sw) logistic regression module of SPSS.

9.10 Training of veterinarians

A two-day-long training was conducted with 10 veterinarians (CDVF veterinarians and Upazila Veterinary Surgeons) of the respective study areas at Department of Medicine, BAU, Mymensingh where PI and CIs were the resource persons.

9.11 Workshop

A day-long workshop was conducted with learned teaching staffs, veterinarians, PhD and MS Fellows, project implementing personnel and BAURES authority at Medicine Conference Hall, Faculty of Veterinary Science, BAU, Mymensingh.

9.12 Formulation of mastitis control strategy

A mastitis control guideline was prepared based on the project findings and recommendation from the workshop.

10. Results and Discussion

10.1 Prevalence of subclinical mastitis

Farm-level prevalence

Total 399 farms were examined, of which 117 (29.3%) had subclinical mastitis (Table 2). The highest farm-level prevalence was recorded in Mymensingh (45.5%) followed by Chittagong (38.6%) and the lowest was in Sirajgonj (9.6%).

Cow-level prevalence

Of the total 931 dairy cows examined, 16.5% (154) had subclinical mastitis. Cross-bred cows were affected at higher rate (17.6%) than indigenous cows (6.5%) (Table 3). The prevalence of SCM was higher (24.5%) in late lactation (>9 months) and lower (14.2%) in early lactation (≤3 months). Older cows (>8 years) were more affected (20.8%) than younger (≤4 years) cows (15.1%). Cows having BCS (≤2) were more (18.0%) susceptible than cows having BCS >3 (11.5%). High yielding cows (above 15) were more affected (25.8%) with SCM than those of low yielding (≤5) cows (12.5%). Cows with 5 and above parity were more (19.8%) susceptible than those of having parity 1-2 (14.6%). In Trishal, Mymensingh, 28 cows from 11 farms were examined which do not represent the population, that is why, the prevalence data for Mymensingh should not be generalized.

Table 2: Farm-level prevalence of subclinical mastitis in smallholder dairy farms

District	No. of farms examined	No. of SCM positive farms	Prevalence (%)
Chittagong	88	34	38.6
Mymensingh	11	5	45.5
Satkhira	248	73	29.4
Sirajgonj	52	5	9.6
Total	**399**	**117**	**29.3**

Table 3: Cow-level prevalence of subclinical mastitis in lactating dairy cows as influenced by age, parity, breed, body condition score, milk yield and stage of lactation

Variable	No. of cows examined	No. of SCM positive cows	Prevalence (%)
District			
Chittagong	380	58	15.3
Mymensingh	28	5	17.9
Satkhira	450	86	19.1
Sirajgonj	73	5	6.8
Breed			
Cross*	839	148	17.6
Indigenous	92	6	6.5
Age (years)			
≤4	285	43	15.1
>4-8	595	101	17.0
Above 8	48	10	20.8
Parity			
1-2	480	70	14.6
3-4	355	65	18.3
5 and above	96	19	19.8
Body condition score			
≤2	205	37	18.0
>2-3	648	108	16.7
Above 3	78	9	11.5
Lactation stage (Month)			
≤3	401	57	14.2
>3-6	275	42	15.3
>6-9	145	28	19.3
Above 9	110	27	24.5
Milk yield (Litre)			
≤5	287	36	12.5
>5-10	372	62	16.7
>10-15	210	40	19.0
Above 15	62	16	25.8

*Crosses of indigenous (zebu) cows with Holstein-Friesian, Jersey, or Sahiwal.

10.2 Risk factors of subclinical mastitis

Cow-level risk factors

Of the 10 variables entered for analysis, seven variables had significant association with SCM in the bivariable analysis (Table 4). Dairy cows with a history of previous clinical mastitis had the strongest point estimate of effect (odds ratio (OR) 36.17) and high statistical significance ($p<0.001$) despite wide 95% confidence interval (CI) of 19.55-66.94 (Table 4). Other factors positively associated with SCM were parity (5 and above), breed (cross), BCS (≤2.5), dirty teat and udder, cylindrical type of teat, pendulous type of udder. Seven variables with $p\leq0.2$ in bivariable analysis were considered for inclusion in the multivariable analysis to estimate the independence of effects of the variables. The final model identified five variables as potential independent cow-level risk factors for SCM of dairy cows (Table 5). These were breed, parity, cylindrical type of teat, pendulous type of udder and history of previous clinical mastitis which had the strongest association with SCM with high statistical significance ($p<0.05$).

Table 4: Bivariable analysis of potential cow-level risk factors for subclinical mastitis in lactating dairy cows

Risk factors	Case cows (n=154) No. (%)	Control cows (n=308) No. (%)	Odds ratio (95% CI)	p-value
Age (years)				
≤4	43 (27.9)	101 (32.8)	Reference	
>4-8	101 (65.6)	193 (62.7)	1.23 (0.79-1.89)	0.347
Above 8	10 (6.5)	14 (4.5)	1.68 (0.69-4.07)	0.253
Parity				
1-2	59 (38.3)	158 (51.3)	Reference	
3-4	66 (42.9)	120 (39.0)	1.47 (0.96-2.25)	0.073
5 and above	29 (18.8)	30 (9.7)	2.58 (1.43-4.67)	0.002
Breed				
Indigenous	6 (3.9)	36 (11.7)	Reference	
Cross	148 (96.1)	272 (88.3)	3.27 (1.35-7.93)	0.009
BCS				
≤2	115 (74.7)	191 (62.0)	2.48 (1.15-5.32)	0.020
>2-3	30 (19.5)	80 (26.0)	1.54 (0.67-3.57)	0.313
Above 3	9 (5.8)	37 (12.0)	Reference	
Lactation stage (months)				
≤3	57 (37.0)	125 (40.6)	Reference	
>3-6	51 (33.1)	104 (33.8)	1.08 (0.68-1.70)	0.756
>6-9	22 (14.3)	39 (12.7)	1.24 (0.67-2.28)	0.494
Above 9	24 (15.6)	40 (13.0)	1.32 (0.73-2.39)	0.366
Milk yield (Litre)				
≤5	36 (23.4)	86 (27.9)	Reference	
>5-10	62 (40.3)	125 (40.6)	1.19 (0.72-1.94)	0.501
>10-15	40 (26.0)	70 (22.7)	1.37 (0.79-2.37)	0.267
Above 15	16 (10.4)	27 (8.8)	1.42 (0.68-2.94)	0.351
History of previous clinical mastitis	100 (64.9)	15 (4.9)	36.17 (19.55-66.94)	<0.001
Dirty teat and/or udder	22 (14.3)	14 (4.5)	3.50 (1.74-7.05)	<0.001
Teat type (cylindrical)	125 (81.2)	216 (70.1)	1.84 (1.15-2.94)	0.012
Udder type (pendulous)	129 (83.8)	200 (64.9)	2.79 (1.71-4.54)	<0.001

Table 5: Results of final multivariable analysis of potential cow-level risk factors for subclinical mastitis in lactating dairy cows

Risk factors	Odds ratio	95% Confidence interval	p-value
Breed (cross)	4.19	1.26-13.93	0.019
Parity			
1-2	Reference		
3-4	1.56	0.88-2.75	0.130
5 and above	2.67	1.11-6.43	0.028
Udder type (pendulous)	3.93	1.98-7.81	<0.001
Teat type (cylindrical)	2.01	1.07-3.77	0.029
History of previous clinical mastitis	44.23	22.04-88.75	<0.001

Farm level risk factors

Of the 28 variables, 17 variables had significant association with SCM in the bivariable analysis (Table 6). Among them floor component (soil) (odds ratio, OR 4.16) and dirty floor conditions (OR 3.33) had significant association with SCM ($p<0.001$). Other factors positively associated with SCM were absence of and insufficient sunlight, age of shed (>5 years), no feeding after milking, manure cleaning once daily, different milking man (i.e. same person does not milk the cows daily), infrequent floor cleaning (once or twice a day), milking by ghosh/gowala, cows not milked sequentially (i.e. sequence in milking of older and young cows, non-infected and infected cows is not followed), no teat dipping during milking, incomplete milking, history of clinical mastitis during last year, no hand wash before milking, wipes udder and teat before milking and cows not grazed during day.

The final model identified 11 variables as potential independent farm-level risk factors for SCM in dairy cows (Table 7). These were absence of and insufficient sunlight, floor component (soil), dirty floor condition, no use of antiseptic after floor cleaning, infrequent floor cleaning (once or twice daily), different milking man, cows not milked sequentially, history of clinical mastitis during last year, cows not grazed during day, wipes udder and teat before milking and age of shed above 5 years, which had the strongest association with SCM with high statistical significance ($p\leq0.05$).

Table 6: Farm-level potential risk factors for subclinical mastitis in dairy cows in bivariable analysis

Risk factors	Case farms (n=117) No. (%)	Control farms (n=117) No. (%)	Odds Ratio (95% CI)	P-value
Insufficient ventilation	30 (25.6)	16 (13.7)	2.18 (1.11-4.26)	0.023
Sunlight				
Absent	48 (41.0)	35 (29.9)	3.33 (1.56-7.12)	0.002
Insufficient	55 (47.0)	48 (41.0)	2.78 (1.34-5.79)	0.006
Sufficient	14 (12.0)	34 (29.1)	Reference	
Floor component (Soil)	40 (34.2)	13 (11.1)	4.16 (2.08-8.39)	<0.001
Dirty floor condition	79 (67.5)	45 (38.5)	3.33 (1.94-5.71)	<0.001
Drain				
Clean	40 (34.2)	45 (38.5)	Reference	
Dirty	39 (33.3)	37 (31.6)	1.19 (0.64-2.20)	0.590
None	38 (32.5)	35 (29.9)	1.22 (0.65-2.35)	0.531
Age of shed above 5 years	54 (46.2)	38 (32.5)	1.78 (1.05-3.03)	0.033
No feeding after milking	67 (57.3)	48 (41.0)	1.93 (1.15-3.24)	0.013

Risk factors	Case farms (n=117) No. (%)	Control farms (n=117) No. (%)	Odds Ratio (95% CI)	P-value
Manure cleaning daily				
Four times	3 (2.6)	7 (6.0)	Reference	
Thrice	32 (27.4)	45 (38.5)	1.66 (0.40-6.91)	0.487
Twice	37 (31.6)	40 (34.2)	2.16 (0.52-8.97)	0.290
Once	45 (38.5)	25 (21.4)	4.20 (1.00-17.74)	0.050
No bath	10 (8.5)	7 (6.0)	1.47 (0.54-4.00)	0.452
Bath water by				
Tubewell	39 (33.3)	42 (35.9)	Reference	
Tap water	38 (32.5)	45 (38.5)	0.91 (0.49-1.68)	0.762
Pond	40 (34.2)	30 (25.6)	1.44 (0.76-2.73)	0.270
No use of antiseptic after floor cleaning	68 (58.1)	57 (48.7)	1.46 (0.87-2.45)	0.150
Frequency of milking				
Thrice	30 (25.6)	37 (31.6)	Reference	
Twice	80 (68.4)	75 (64.1)	1.32 (0.74-2.34)	0.350
Once	7 (6.0)	5 (4.3)	1.73 (0.50-5.99)	0.390
Different milking man	70 (59.8)	50 (42.7)	1.91 (1.19-3.36)	0.009
Floor cleaning times daily				
Once	42 (35.9)	25 (21.4)	3.36 (1.52-7.43)	0.003
Twice	60 (51.3)	62 (53.0)	1.94 (0.95-3.95)	0.070
Thrice	15 (12.8)	30 (25.6)	Reference	
Milking by whom				
Labor	32 (27.4)	38 (32.5)	1.13 (0.60-2.15)	0.708
Milking man (Ghosh/Gowala)	50 (42.7)	32 (27.4)	2.10 (1.13-3.91)	0.020
Self	35 (29.9)	47 (40.2)	Reference	
Cows not milked sequentially	67 (57.3)	49 (41.9)	1.90 (1.11-3.12)	0.019
Calves allowed to suck milk after milking	57 (48.7)	65 (55.6)	1.32 (0.79-2.20)	0.296
No sufficient floor space	54 (46.2)	45 (38.5)	1.37 (0.82-2.31)	0.234
Distance of manure pit from shed				
>10 ft				
>5-10 ft	24 (20.5)	32 (27.4)	Reference	
≤5 ft	27 (23.1)	25 (21.4)	1.44 (0.67-3.16)	0.346
	66 (56.4)	60 (51.3)	1.47 (0.78-2.71)	0.237
No teat dipping during milking with antiseptic	65 (55.6)	45 (38.5)	2.00 (1.19-3.37)	0.009
Incomplete milking	68 (58.1)	52 (44.4)	1.74 (1.03-2.91)	0.037
History of clinical mastitis during last year	48 (41.0)	30 (25.6)	2.02 (1.16-3.51)	0.013
No farmers training	102 (87.2)	97 (82.9)	1.40 (0.68-2.89)	0.361
No hand wash before milking	67 (57.3)	47 (40.2)	1.99 (1.19-3.36)	0.009
Wipes udder and teat before milking	24 (20.5)	11 (9.4)	2.49 (1.16-5.40)	0.020
Position shed				
East-West	56 (47.9)	63 (53.8)	1.27 (0.76-2.12)	0.360
North-South	61 (52.1)	54 (46.2)	Reference	
Others animal and poultry near shed	47 (40.2)	40 (34.2)	1.29 (0.76-2.20)	0.344
Cows not grazed during day	69 (59.0)	52 (44.4)	1.80 (1.07-3.02)	0.027

Table 7: Farm-level potential risk factors for subclinical mastitis in dairy cows in final model of multivariable analysis

Risk factors	Odds Ratio	95% CI	P-value
Sunlight			
Absent	8.23	2.16-31.41	0.002
Insufficient	4.17	1.11-17.91	0.055
Sufficient	Reference		
Floor component (Soil)	6.23	2.10-18.46	0.001
Dirty floor condition	21.77	7.04-67.34	<0.001
No use of antiseptic after floor cleaning	2.48	1.02-6.01	0.045
Different milking man	8.08	2.40-27.21	0.001
Floor cleaning times daily			
Once	18.19	3.35-98.83	0.001
Twice	7.30	2.10-25.37	0.002
Thrice	Reference		
Cows not milked sequentially	3.06	1.14-8.19	0.026
History of clinical mastitis during last year	3.2	1.29-7.98	0.012
Wipes udder and teat before milking	6.72	1.87-24.10	0.003
Cows not grazed during day	2.44	1.02-5.82	0.045
Age of shed above 5 years	4.91	1.92-7.98	0.001

10.3 Results of bacteriological analysis

As major pathogens, coagulase positive *Staphylococcus aureus* and *E. coli* were identified only in the SCM positive milk samples (Table 8, Fig. 5-7). Coagulase negative *Staphylococcus aureus* (CNS) and *Bacillus* spp. were detected in both SCM positive and negative samples, of them, CNS were most frequent (Table 8, Fig. 8).

Table 8: Isolation and identification of bacterial species and relating that with SCC

SCM	SCC ($\times 10^3$/ml)	Major pathogens		Minor pathogens		No growth
		Coagulase Positive *Staph. aureus*	*E. coli*	CNS	*Bacillus* spp.	
-ve	≤200 (n=50)	-	-	05	1	44
+ve	>200-600 (n=22)	03	3	20	2	2
	>600-900 (n=16)	10	5	16	1	-
	>900 (n=14)	14	7	14	-	-

CNS = Coagulase negative *Staphylococcus* spp.

10.4 Workshop

In a day-long workshop, the UGC Professor Dr. Muzahed Uddin Ahmed and Professor Dr. Lutful Hassan, Director of BAURES were the chief guest and special guest, respectively. The Dean of the Faculty of Veterinary Science, BAU, Mymensingh chaired the workshop. Following presentation on project activities and findings, participants were divided into four groups for discussion and to have recommendation on prevention and control of mastitis in dairy cows based on the project findings. Then, each group presented their group's recommendation and finally two rapporteurs compiled those (Fig. 9-12).

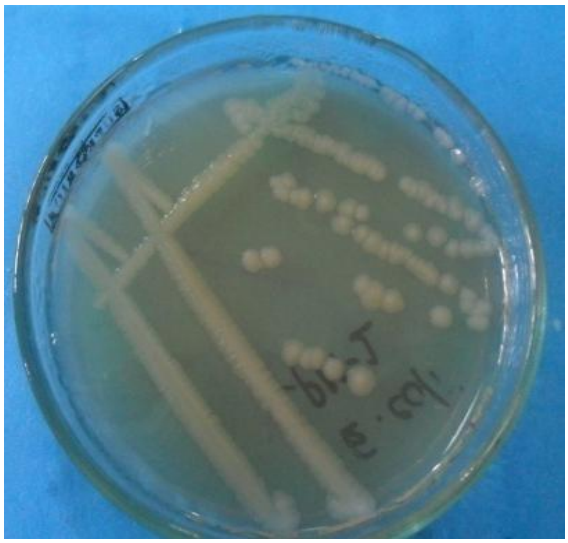

Fig. 5. Colony of *E. coli* showing smooth, circular, white colony on nutrient agar.

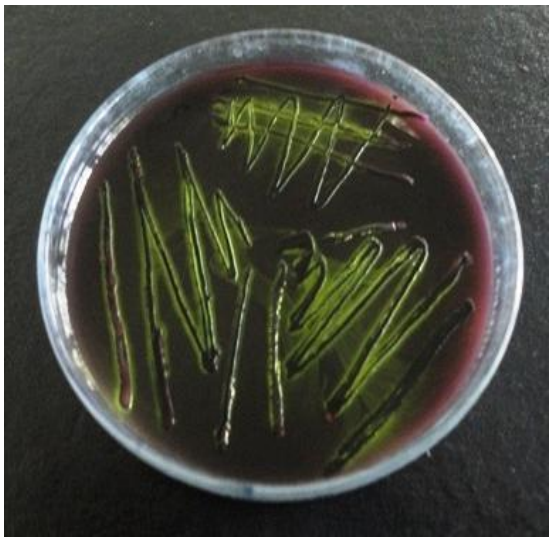

Fig. 6. Colony of *E. coli* showing metallic sheen on eosin methylene blue agar.

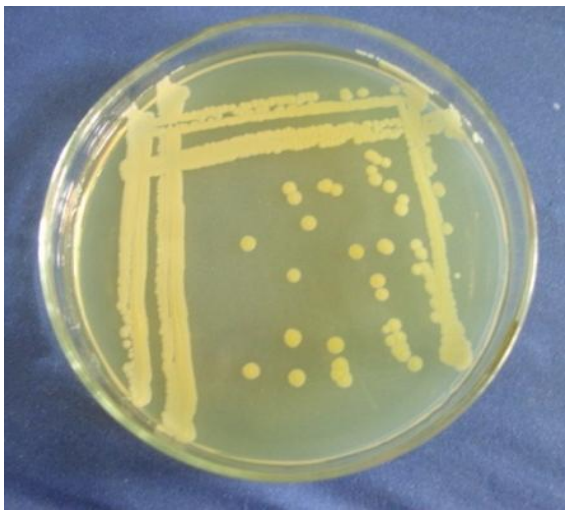

Fig. 7. Colony of Staphylococcus spp. producing gray white colony on nutrient agar.

Fig. 8. Thick, creamy colored colonies of *Bacillus* spp. on nutrient agar.

Fig. 9. UGC Professor, the Chief Guest of the workshop is addressing.

Fig. 10. The PI of the project is presenting the project activities and findings.

Fig. 11a-b. Group discussion to have recommendations on prevention and control of bovine mastitis.

Fig. 12. Group presentation on recommendations to prevent and control bovine mastitis.

10.5 Formulation of mastitis control strategy

Based on the project findings and recommendation from the workshop, a mastitis control guideline was prepared that was published in the form of booklet entitled "**গাভীর ওলানফোলা রোগের পরিচিতি ও নিয়ন্ত্রণ নির্দেশিকা**". A leaflet was also prepared on this issue. Both the booklet and leaflet were distributed to the smallholder dairy farmers of the project areas as well as to the faculties. However, control strategies stated in the booklet should be validated for generalized use of it.

11. Research Highlights (bullet points-max.10 nos.):

- Adoption and standardization of somatic cell count in milk samples
- Early diagnosis of subclinical mastitis (SCM) by using automatic somatic cell counter
- Identification of major and minor pathogens of SCM
- Identification of farm-level and cow -level risk factors for SCM in dairy cows
- Improvement of skills on dairy cattle health and production management of the veterinarians by training
- Formulation of mastitis control guidelines

12. Major Attainments *(in relation to the set objectives)*:

a. Technical : Output, Outcome and Impact

Sl. No.	**Major technical activities performed in respect of the set objectives**	**Output (i.e. product obtained, visible, measurable)**	**Outcome (short term effect of the research)**	**Impact (long term effect of the research)**	**Remarks (reason, if anything otherwise plus any other)**
1	Adoption and standardization of somatic cell count in milk samples	Early diagnosis of subclinical mastitis (SCM) in lactating dairy cows by automatic somatic cell counter	Farmers can get the diagnostic service at minimum cost	Losses from SCM will be reduced	
2	Bacteriological analysis (cultural and biochemical) of milk samples	Major and minor pathogens causing SCM are identified and correlated with somatic cell count	SCM causing pathogens are known to researchers and farmers in Bangladesh context	Will help to select appropriate antibiotic for treatment purpose	
3	Farm investigation for cross-sectional and case-control studies and analysis of data using advanced statistical techniques	Prevalence and risk factors of SCM in lactating dairy cows are known	Farmers and researchers gain knowledge about the risk factors related to SCM	Knowledge on risk factors will help the farmers for better farm management practices that will reduce prevalence of SCM	
4	Preparation of mastitis control guidelines (Booklet and leaflet)	Booklet and leaflet on prevention and control of mastitis are in place*	Farmers are aware and well known on prevention and control of mastitis in dairy cows	Better management of the dairy cows; prevalence of mastitis will be reduced and losses will be minimized	

*Control strategies stated in the booklet were formulated based on the findings of risk factors and recommendation from the workshop, and should be validated for generalized use for the farmers.

b. Procurement

Sl. No.	Approved provisions of Procurement (list major items)	Achievement	% of achievement	Remarks (statement on the handing over of the materials procured/developed as per LoA plus any other)
1	Procurement of Media	Completed	100%	
2	Procurement of Chemicals & reagents	Completed	100%	
3	Procurement of Appliances	Completed	100%	
4	Procurement of Vehicle Hire	Completed	100%	
5	Procurement of Printing and Publication	Completed	100%	
6	Procurement of somatic Cell count	Completed	100%	
7	Procurement of repair & renovation	Completed	100%	

c. HRD/ Training

Title (e.g Ph.D/MS/ Trainings, workshops conducted etc.)	Target	Attain-ments	No. of participants	Benefit of the higher studies/trainings(application of the learning, productivity enhancement	Remarks (reason, if anything otherwise)
MS thesis title 1. Identification, molecular detection and antobiogram profile of bacteria isolated from California mastitis test positive milk samples of crossbreed cows of Satkhira district. 2. Comparison between California mastitis test and Somatic cell count as diagnostic tools for Bovine subclinical mastitis.	2	2	2	Two students awarded MS degree	
Training on Dairy Cattle Health and Production Management	1	1	10	Skills on dairy cattle health and production management of the veterinarians improved	
Workshops on Prevention and Control of Mastitis in dairy cows	1	1	100	Guidelines for the prevention and control of mastitis in dairy cows formulated and distributed to the farmers	

d. Financial

Sl. No.	Major Head	Fund received (Tk.)	Expenditure (Tk.)	Balance/Unspent (Tk.)	Remarks (reason, if anything otherwise)
	Salary & Remuneration	6,97,716.00	6,97,716.00	0.00	
	Research expenses	16,48,726.00	16,48,624.00	102.00	
	Operating expenses	1,87,479.00	1,87,479.00	0.00	
	Vehicle hire, Fuel, Oil and Maintenance	1,54,500.00	1,54,500.00	0.00	
	Training/Workshop/Seminar etc.	1,90,350.00	1,90,350.00	0.00	
	Publications & printing	1,48,500.00	1,48,500.00	0.00	
	Contingencies	44,969.00	45,071.00	-102.00	Bank charge*
	Capital expenses	16,24,400.00	16,24,400.00	0.00	
	Total	46,96,640.00	46,96,640.00	0.00	

*Unspent money of Tk 102 in the Research expenses item included in the contingencies as bank charge.

e. Materials developed/Publications made

Type of material/publication	Title	Number	Remarks (being used by/meant for/any other)
Technology development	Early detection of subclinical mastitis by automatic somatic cell counting	1	
Booklet/leaflet/flyer etc published a. Leaflet b. Booklet	a.গাভীর ওলানফোলা রোগ ঃ প্রতিরোধ ও প্রতিকার b.গাভীর ওলানফোলা রোগের পরিচিতি ও নিয়ন্ত্রণ নির্দেশিকা	a. 2000 b. 2000	

13. Sub-project Auditing (cover all types of audit performed)

Types of Audit (e.g BARC/Implementing agency/FAPAD/World Bank/others)	Major observations/issues/objections raised, if any	Status at the sub-project end	Remarks
FAPAD	None		
BARC (J.U. Ahamed and Co.)	None		

14. Reporting

Report type	Actual date of submission(s)	Total Number(s)	Remarks (if anything otherwise)
a. Inception report	03.10.12	01	
b. Monthly reports*	08.12.12; 08.01.13; 11.02.13; 06.03.13; 04.04.13; 15.05.13; 09.06.13; 17.07.13; 28.09.13; 08.10.13; 09.10.13; 11.12.13	12	

Report type	Actual date of submission(s)	Total Number(s)	Remarks (if anything otherwise)
c. Statement of expdts. (SoE)*	08.12.12; 08.01.13; 11.02.13; 06.03.13; 04.04.13; 15.05.13; 09.06.13; 17.07.13; 28.09.13; 08.10.13; 09.10.13; 11.12.13	12	
d. Quarterly report(s)*			
e. Six monthly report	08.10.13	01	
f. Procurement plan	04.11.12; 21.08.13	02	
g. Annual research program format	08.10.13	01	
h. Environmental monitoring (Annual Basis)			
i. Social safeguard status (Before and at the end)			
j. Field Monitoring Report(s)**			

** Provide all since start to end, ** Conducted at the local level by implementing agencies.*

15. Problems/Constraints (bullet points- max. 5 nos.) :

Delay in the approval of procurement plan; timely procurement of research materials was not possible due to system fault ; some farmers sought incentive.

16. Suggestion for future, if any :

Workshop with the project personnel on the key highlights on project management policy and PI's responsibilities immediately before or after the signing of LoA

Signature of the Coordinator/Principal Investigator (as applicable)

Date: 29 June 2014

Seal

Dr. Md. Taohidul Islam
Principal Investigator
Somatic cell count.....Mastitis
Department of Medicine
Bangladesh Agricultural University
Mymensingh-2202, BANGLADESH

Counter signature of the Head of the agency/authorized representative

Date: 29 June 2014

Seal

Prof. Dr. Lutful Hassan
DIRECTOR
BAU Research System (BAURES)
Mymensingh-2202,BANGLADESH
Mobile: 01730-712055 Ph: 091-67471
Email:baures84@gmail.com

Annex-I

Somatic Cell Count and Bacterial Characterization as Tools for Diagnosis and Identification of Risk Factors of Bovine Mastitis

Questionnaire: 1 (Farm Level Information)

Farm ID:	Farmer's name:	Date:
Village:	Upazila:	District:
Farmer's Training: Yes/ No Specify: Keep other animals/poultry near the shed: Yes/No Specify: Herd size: ➢ Lactating cow: ➢ Dry cow: ➢ Pregnant heifer: ➢ Non pregnant heifer: ➢ Calves: ➢ Bull: **Housing Information:** No. of shed: 1/2/3/4 Age of Shed: Shed 1. Shed 2. Shed 3. Shed 4. Position of shed: East-West(shed-1/2/3/4) North-South(shed-1/2/3/4)	Floor area (ft): Length: Width: Shed 1. Shed 2. Shed 3. Shed 4. No. of cows on the floor: Shed 1. Shed 2. Shed 3. Shed 4. Roof material: 1. 2. 3. 4. Ventilation: 1=Natural (Shed 1/2/3/4) 2=Fan system (Shed 1/2/3/4) 3= Both (Shed 1/2/3/4) Shed gets sunlight: (Shed 1/2/3/4) Floor Cleaning: .....Daily/weekly Floor cleaning by: water/antiseptic solution	Floor component: Concrete (Shed 1/2/3/4) Brick block without cementing (Shed 1/2/3/4) Brick block with cementing (Shed 1/2/3/4) Soil only (Shed 1/2/3/4) Floor condition: Dry & Clean (Shed 1/2/3/4) Wet& Clean (Shed 1/2/3/4) Dry & soiled with dung (Shed 1/2/3/4) Wet & soiled with dung (Shed 1/2/3/4) Drain: Present or absent, if present 1=Dry & clean(Shed 1/2/3/4) 2=Dry & dirty(Shed 1/2/3/4) 3=Wet & clean(Shed 1/2/3/4) 4=Wet & dirty(Shed 1/2/3/4) Manure pit close to the farm: Yes/No Manure cleaning: .....times daily/weekly Total no. of labor in farm:

Feeding practice: Cows on pasture by day: yes/no Cows tethered in the barn by day: yes/no **Feeding schedule:** Morning: before milking/after milking Afternoon: before milking/after milking **Cow's bath practice:** Bathed: yes / no, ...times daily/weekly/monthly Bath water: Tube well /tap water/pond/river	**Milking practice:** Frequency: 1/2/3/4 Milking by: Hand/Machine Milking by whom: Self/Labor/Milking man (Gosh/Gowala) Same person milking daily: yes/no Milking sequence: yes/no **Milking hygiene practice:** Wash udder and teat before milking: yes/no If yes- antiseptic soln/water Wash udder and teat after milking: yes/no If yes- antiseptic soln/water	Wipes udder and teat before milking: yes/no If yes, pls specify …….. Teat dipping during milking with antiseptic: yes/no If yes- Before/after/both Specify names of dipping agents: Udder spray: yes/no If yes- antiseptic soln/water Fore milk discarded: yes/no
Mastitis related information: Total no. of mastitis affected cows last year: Total lactating cows last year:	How mastitis was treated:	Who treated the mastitic cows: Quack/Veterinarian/Self No. of mastitic cows cured after treatment:

Questionnaire: 2 (Lactating cow information)

Cow ID: Identification mark/name: Breed: Age: Lactation stage: Calving date: Parity: Milk production: L/day	**Last puerperium:** 1= Normal 2= Vaginal prolapse 3= Uterine prolapse 4=Milk fever 5=Retained placenta 6= Other abnormalities	Body weight: BCS: **Udder cleanliness:** 1= Clean udder and teat 2=Clean udder and dirty teat 3=Dirty udder, clean teat 4=Dirty udder and teat History of previous mastitis:

Udder and milk examination:		
Udder position: Pre-inguinal / inguinal / post-inguinal **Udder shape:** Pendulous /Round (bag)/Bowl / bottle **Teat shape:** Conical/Funnel/Cylindrical/ Platform **Udder lesions:** yes/no Pls. specify: **Teat Lesions:** yes/no Pls. specify: Supernumerary teat: present/absent, if present – specify	**Clinical findings:** 1=Normal udder and milk 2=Normal udder but flakes/clot in milk 3=Normal udder but little or no milk 4=Udder swollen, warm and milk watery 5=Udder hard and milk clotted 6=Udder hard, painful and blood in milk 7=Udder atrophied and no milk	**Clinical Diagnosis:** 1=Normal udder 2=Mild/moderate/severe mastitis 3=Udder trauma 4=Udder edema 5=Teat injury 6=Others **Who treated the present mastitic cow (if any)?** Quack/Veterinarian/Self Treatment history of the present case:

CMT score	**Udder palpation score**	**Milk score**
Right Front= Left Front= Right Rear= Left Rear= 1=Normal 2=begins to coagulate 3=coagulate, does not sticks 4=coagulates and sticks **SCC** Right Front= Left Front= Right Rear= Left Rear=	Right Front= Left Front= Right Rear= Left Rear= 1=no swelling and pain 2=ventral quarter swollen 3=generalized swollen quarter 4=swollen and painful	Right Front= Left Front= Right Rear= Left Rear= 1=Normal 2=flakes/clots otherwise normal milk 3=little/no milk; moderately abnormal color 4=no normal milk; watery, serum or blood

Mastitis Severity Scoring (will be scored by the vet based on above findings)

Mastitis	CMT	Udder	Cow
Mild	2-3	2	Normal
Moderate	3-4	3	Normal
Severe	3-4	3	2 or more abnormal parameter

References

Chowdhury MK, 2011. Epidemiology of bovine mastitis in Bangladesh. PhD Thesis, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.pp.88

Dohoo IR, Martin SW, Stryhn H, 2003. Veterinary Epidemiological Research. AVC Inc., Charlottetown, Prince Edward Island, Canada.

Halasa TK, Huijps K, Osteras O, Hogeveen H, 2007. Economic effects of bovine mastitis and mastitis management: A review. Vet Q 29:18-31.

Hogan JS, Gonzalez RN, Harmon RJ, Nickerson SC, Oliver SP, Pankey JW, Smith KL, 1999. Laboratory and field handbook on bovine mastitis. Madison, WI, National Mastitis Council, Inc, p 1-33.

Hogeveen H, Huijps K, Lam TJ, 2011. Economic aspects of mastitis: new developments. N Z Vet J 59:16-23

Hosmer DW, Lemeshow S, 2000. Applied Logistic Regression. 2nd ed. John Wiley and Sons, Toronto, Canada.

Seegers H, Fourichon C, Beaudeau F, 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet Res 34:475-491.